Exhibit F-15
Appl. No. 09/558,421

Project N _____
B k No._____

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Page N ___

PEI + AS $(NH_4)_2SO_4$ ppt.

an to continue with purification - Allowing the
ime protocal as in wild type Tne - p.108.0

11 - 6.8 mL        $(.05)(6.8) = 2M \cdot x$        $x = 174 \mu l$ q 2M KCl
                        3.8

5'exo - 4.8 mL 3.8     $(.05)(4.8 + x) = 2M x$       $x = 97.4 \mu l$ q 2M KCl
                        7.0

                $(.40\%)(6.8 + x) = 10\% \cdot x$
                $291 \mu l = x$                    $x = 291 \mu l$ 10% DEI

                $(.40)(3.9 + x) = 10\% \cdot x$
                $163 \mu l = x$                    $x = 143 \mu l$ q 10% DEI

Make each a final    50 mM KCl slowly add PEI a 10%
PEI sol'n to a final [] of .4%.   vortex - let shake
30 minutes @ 4°C. spin in 2mL eppendorfs in micro-
centrifuge 20 minutes @ 4°C - Save Supernatant.

        60% $(NH_4)_2SO_4$ fractionation

    Tne I    $\frac{36g \ solid}{100 mL} = \frac{x}{4.8 mL}$    2.45 g

    3'-5 exo-  $\frac{36 g}{100 mL} = \frac{x}{3.5 mL}$    1.26 g

        vortex - let shake 30 min @ 4°C
        spin in SS-34 - 20,000 x g - -
        Decant + Save Supernatant - pellets

To Page No.____

**Project No.** _____
**Book No.** _____   **TITLE** 2mL Toso Heparin.

From Page No. ___

Bump Heparin with .5 M NaOH - Wash w/ H₂O extensive
Equilibrate w/ Buffer A.

Buffer A - Heparin -                    Buffer B - Heparin

25mm Tris pH 7.4                        25mm Tris pH 7.4
10% glycerol                            10% glycerol
5 mm Bme                                5 mM Bme
.1 mM PMSF                              .1 mM PMSF
.1 mM EDTA                              .1 mM EDTA
10mM KCl                                1.5 M KCl
conductivity - 1.2 mS

                    A.S.
Ty-1 - Dissolve Pellet in 10mL  of Buffer A        4.5 mS - condu

        Add 30 mL additional of Buffer A          2.1 mS - condu


Load 9 35mL on 2mL Toso Heparin @ .75mL/min
collect flow through material - wash to base line -

        Gradient Program -    0 - 100% B @ .5mL/min - 20mL linear g
                              wash 100% B - 10mL - @ .5mL/min
                              collect 500 µL fractions -
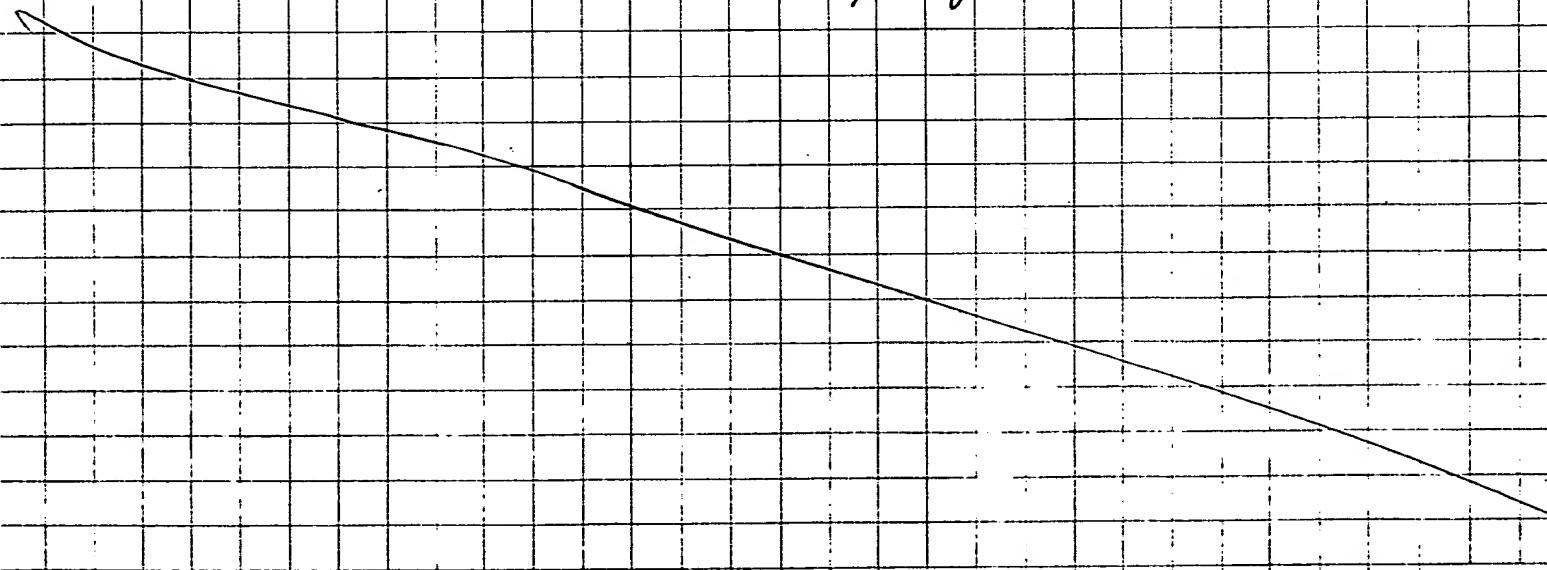
To Page N

| Witness d & Understood by me, | Dat | Inv nted by | Dat |
|---|---|---|---|
| Maun Longo | 6/20/25 | Rec rd d by | 6/15/9 5 |

Page N .___                                                    6/15/95

Mix Rxn

stock                          For 20 mL
.5M TAPS                          1 mL
50 mM MgCl₂                     800 μL
2M KCl                          500 μL
1 M DTT                         200 μL
10 mM dNTP's                    400 μL
ct. Salmon testes               5 mL
                                12.
                               20 mLs        — 1.1 mL dCTP
                                                   /vial

Aliquot 500 μL / tube   Store in -20°C freezer - yellow
                                                     tubes -

| ssed & Understood by me, | Date | Invent d by | Date |
|---|---|---|---|
| May Longs | 6/20/95 | Recorded by | 6/16/95 |

Project No. _____
Book No. _____ TITLE Heparin - FY-1

From Page No. __

06/15

| SAM | CPM1 | | | ΣX | FY-1' |
|---|---|---|---|---|---|
| 1 | 115552.00 | 52 | | | |
| 2 | 53328.00 | 54 | | | |
| 3 | 9146.00 | 56 | | | |
| 4 | 4556.00 | 58 | | | |
| 5 | 1260.00 | 59 | | | |
| 6 | 3744.00 | 60 | | | |
| 7 | 1028.00 | 61 | | | |
| 8 | 574.00 | 62 | | | |
| 9 | 536.00 | 63 | | | |
| 10 | 346.00 | 64 | | | |
| 11 | 730.00 | 65 | | | |
| 12 | 438.00 | 66 | | | |
| 13 | 348.00 | 67 | | | |
| 14 | 21268.00 | Load | | | |
| 15 | 668.00 | 44 | | | |
| 16 | 372.00 | 46 | | | |
| 17 | 866.00 | 48 | | | |
| 18 | 74836.00 | 50 | | | |
| 19 | 146.00 | | | | |

Pool 49-55 dialyze O/N in
Qu50 Buffer A
MY 6/20/95

60

70

80

90

2G447 100

Pharmacia LKB Biotechnology

24 µl Rxn
1 µl fact
sample -
incubate @
fn 8' - qu
w/ 10 µl of 5
EDTA - 5µ
20 µl on 6
wash

5' 1x 10% TC
3' 3x 5% T
2+ Σ to
dry + cou
ecoroflour

Pool - 49
dialyze O/
in again
Qu50 Buf
see p. 144

6/20/55

| Witnessed & Understood by me, | Date | Invented by S. Flynn | Date |
|---|---|---|---|
| May Longo | 6/20/95 | Recorded by | 06/14/95 |

Heparin 3-5 exo mutant

Project No. _____
Book N . _____

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age No. ___

06/15

0806/N50/98109/8.93

The 3-5 exo mutant

| SAM | CPM1 | |
|---|---|---|
| 1 | 266.00 | 20 |
| 2 | 324.00 | 23 |
| 3 | 1126.00 | 24 |
| 4 | 24684.00 | 26 |
| 5 | 33768.00 | 28 |
| 6 | 111394.00 | 30 |
| 7 | 78652.00 | 32 |
| 8 | 29724.00 | 34 |
| 9 | 8666.00 | 36 |
| 10 | 54.00 | 18 |
| 11 | 2912.00 | 38 |
| 12 | 1402.00 | 40 |
| 13 | 13900.00 | Load |
| 14 | 212.00 | B |

Pool -
25-35

MY
6/20/95

27

24 µl mix
1 µl fraction
sample -
incubate @
74°c 8min -
quench w/
10 µl 0.5M
EDTA -
spot 20 µl
on GF/C
wash -
1x 10% TCA
1% P.i
3x 5% TCA
2x EtOH
dry +
count -

Pool 25-35
dialyze 4hrs
in Q150
Buffer A →
see p. 14?
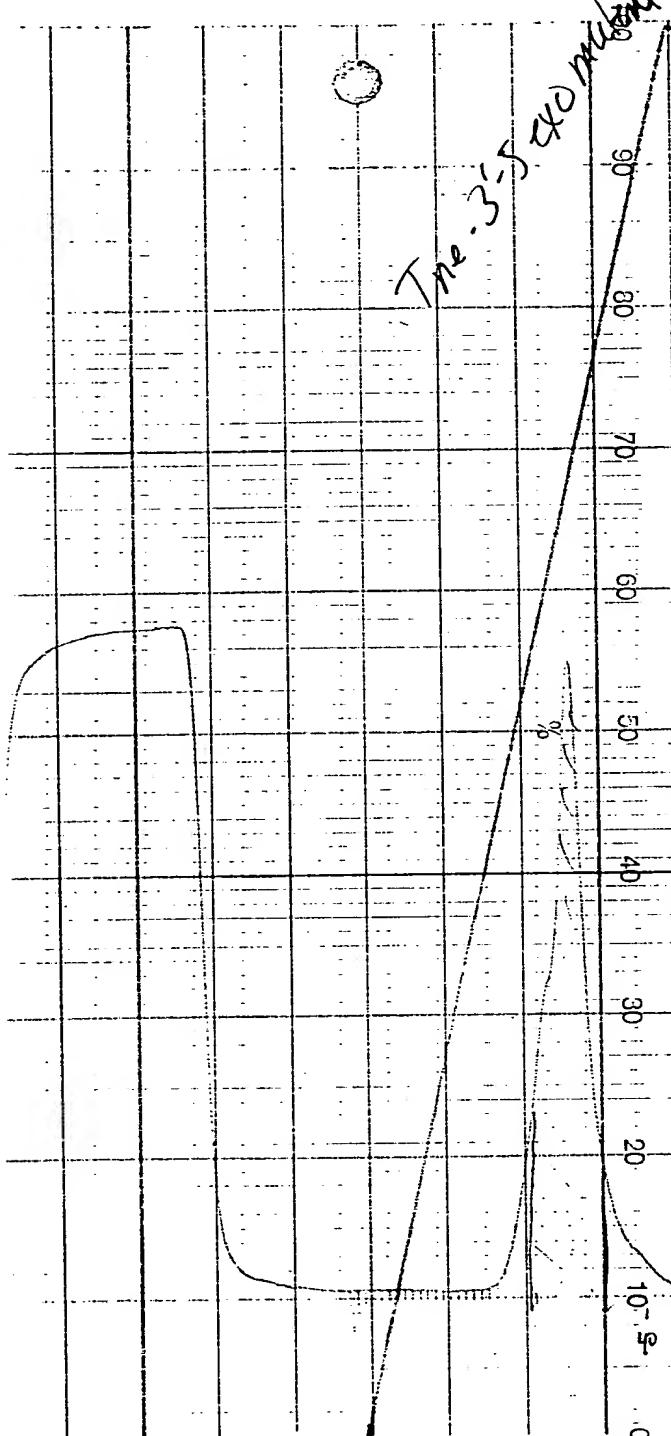
echnology        Code No. 18-1001-44

MY
6/20/95

ET 06/15/95

| sed & Understood by me, | Date | Inv nt d by Elizabeth Ty | Date |
|---|---|---|---|
| Mary Tongo | 6/20/95 | Recorded by | 06/16/95 |